

**Chemosystematics of *Juniperus*: Effects of leaf drying on the essential oil composition of *Juniperus pinchotii*, changes during the first 48 hours of drying**

**Robert P. Adams**

Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA  
email Robert\_Adams@baylor.edu

**ABSTRACT**

A bulk collection of terminal branchlets was made from *J. pinchotii* and subjected to drying at 42-45° C for up to 48 hrs. The oils were distilled and analyzed from fresh leaves and those dried for 4h, 8h, 12h, 24h and 48h. Sabinene,  $\alpha$ -terpinene, cis-sabinene hydrate, camphor, citronellol and bornyl acetate declined during drying, whereas borneol increased. The largest changes occurred between 24 and 48 h of drying (as the leaves became brittle), which explains the previous report of changes in oils between fresh and 0.5 mo. (Adams 2013).

Published on-line: [www.phytologia.org](http://www.phytologia.org) *Phytologia* 95(2): 132-137 (May 1, 2013).

**KEY WORDS:** *Juniperus pinchotii*, oils from dried leaves, chemosystematics, terpene decomposition.

Recently, I reported (Adams, 2013) on changes in volatile leaf oil composition from *Juniperus pinchotii* leaves air dried (42-45°C, 24h) and stored at room temperature (22° C, RT) for up to 24 mos. The oil yields showed a slight decline initially, but remained fairly constant (Fig. 1). Camphor, camphene hydrate and citronellal declined (mg/g dry foliage) in fresh vs. 0.5 mo. samples. Borneol increased during storage (on a mg/g basis). This may be due to the loss of acetate by bornyl acetate and/or oxidation of terpenes to produce borneol.

The major trend (Adams, 2013) was the decrease in camphor, camphene hydrate, and citronellal during drying (fresh vs. 0.5 mo., Fig. 2). In addition, there was a decrease in bornyl acetate and an increase in borneol during the 24 mo. storage at RT. Overall, most of the changes occurred during the drying process, ie. fresh and 0.5 mo. samples. Because the purpose of that study was to examine oil stability *in situ* over a long period (24 mo.), no detailed information was gathered concerning oil changes during the drying process (0 - 48h).

The purpose of this study is to report on the initial changes in composition of leaf oil from *J. pinchotii* leaves during the first 48 hr of drying at 42-45°C. The literature was recently reviewed (Adams, 2013).

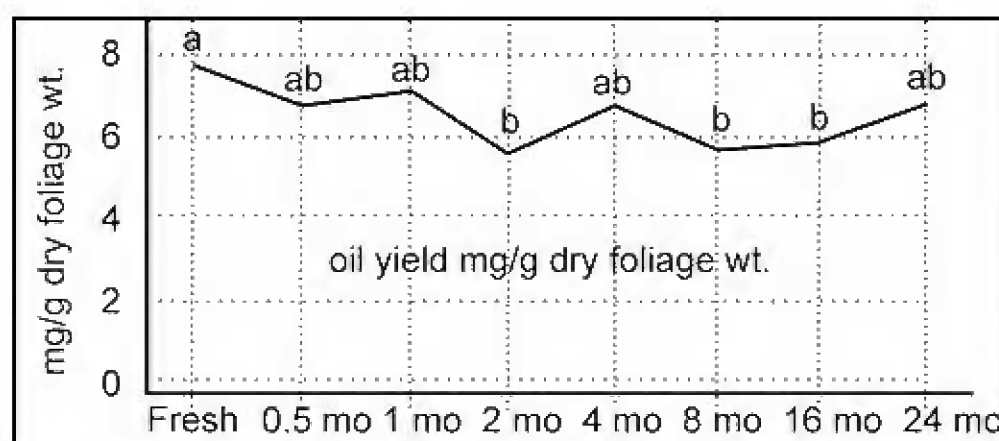


Figure 1. Variation in oil yield (mg/g) from specimens stored at RT for up to 24 mo. Samples sharing a common letter are not statistically different (P=0.05). (from Adams, 2013).

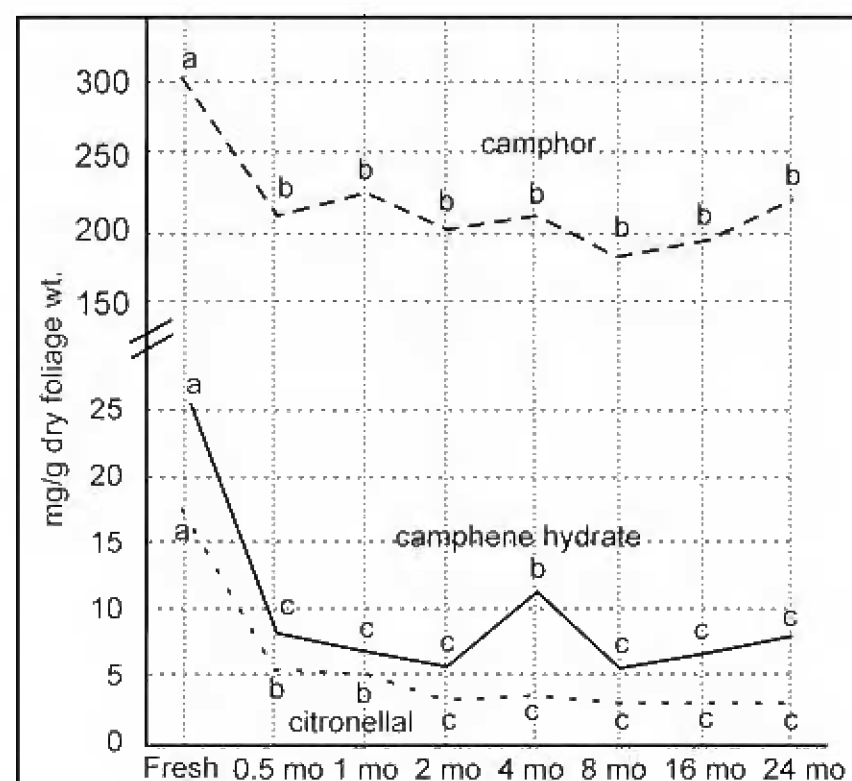


Figure 2. Variation in camphor, camphene hydrate and citronellal

## MATERIALS AND METHODS

**Plant material** - *J. pinchotii*, Adams 13742, 14.2 mi. s of Claude on Tex 209, Armstrong Co., TX. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

**Isolation of oils** - Fresh (200 g) and air dried (100 g) leaves were co-steam distilled with 2 mg of methyl decanoate (internal standard) for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

**Analyses** - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967). Principal component analysis (PCA) follows Veldman (1967).

## RESULTS AND DISCUSSION

Comparisons of the leaf components (on a mg/g foliage oven dry weight basis) from the leaves of *J. pinchotii* from fresh vs. air dried (42 - 45° C) for different periods are shown in Table 1. There is a significant drop in oil yield between 24 and 48h (Table 1, Fig. 3). It should be noted that the plant press was opened to remove a newspaper with pressed leaves at each sampling interval. At 24 h, some of the leaves were still obviously not dried enough for storage (i. e. brittle). After 48 h all the leaves were brittle (i.e., break when bent). It appears that this is the point where most chemical changes occur. It should be noted that this is also the point where one would remove the specimens for herbarium storage. Drying for less time would be insufficient to prevent mold growth on specimens. The increase in oil after 8 h (Fig. 3) might be due to the hydrolysis of terpene glycosides making some terpenes more available for removal by steam distillation.

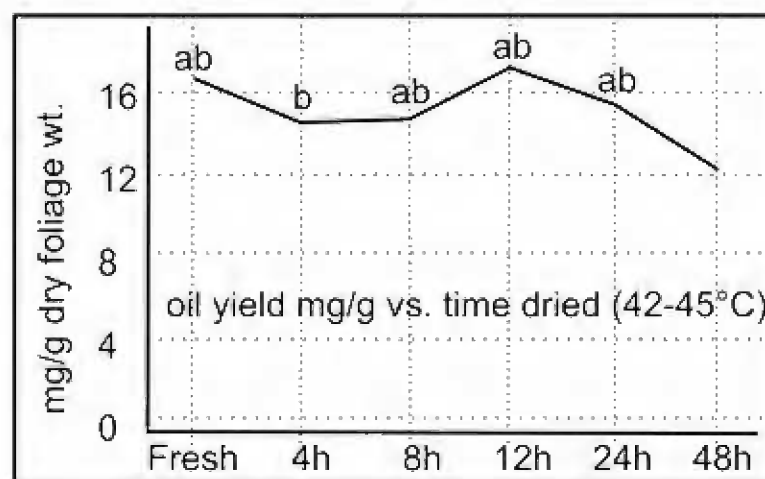


Figure 3. Oil yield vs. time drying. Data points with a common letter are not significantly different by SNK range tests.

Several of the volatile leaf oil components exhibit highly significant declines during drying: sabinene,  $\alpha$ -terpinene, cis-sabinene hydrate, camphor citronellol and bornyl acetate (Table 1). These components exhibited a decline between fresh and 4 h, an increase from 8 to 12 h, then a decline from 12 to 48 h, with the major decline between 24 and 48 h when the leaves become brittle (Fig. 4). Camphor and sabinene (the major oil components, accounting for about 80% of the leaf oil) have a very similar pattern (Fig. 4). It seems likely that the overall loss of oil (see Fig. 3) is a factor in the decline of these two major components.



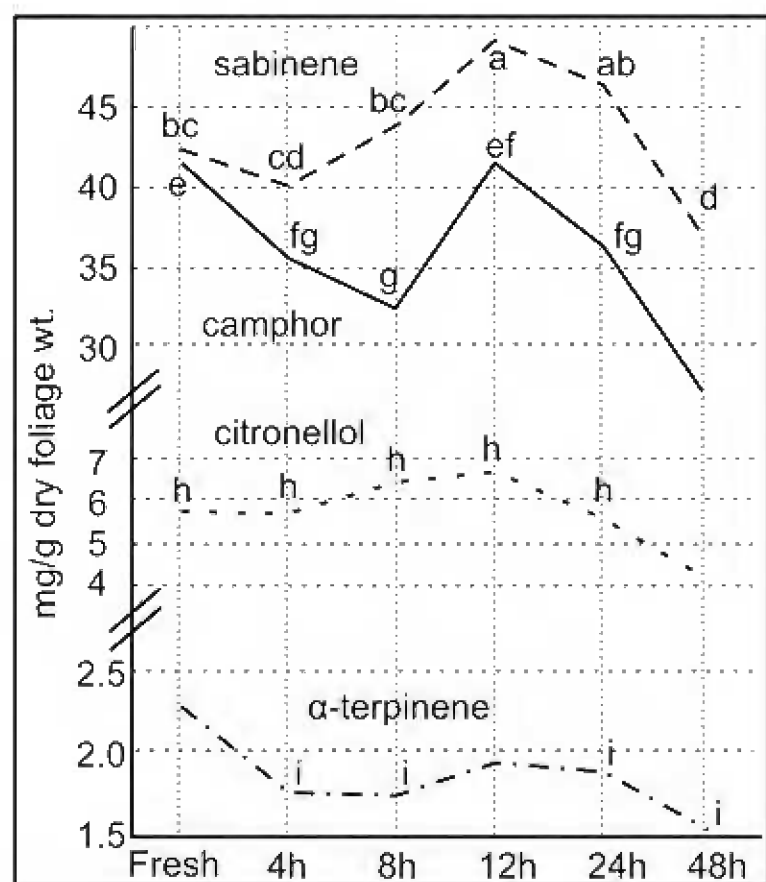


Figure 4. Changes in sabinene, camphor, citronellol and  $\alpha$ -terpinene during drying.

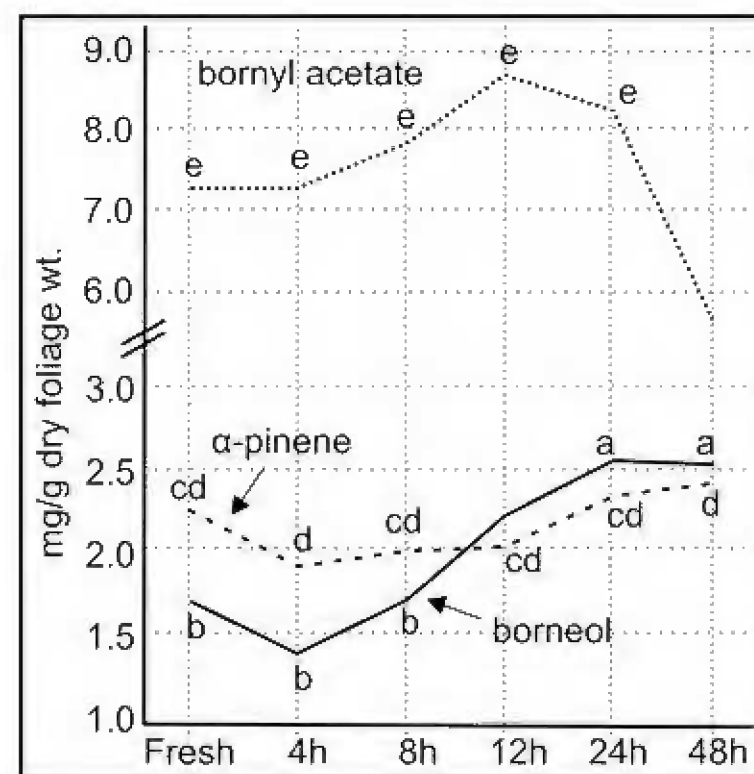


Figure 5. Changes in bornyl acetate, borneol and  $\alpha$ -pinene during drying.

Borneol and to a lesser extent,  $\alpha$ -pinene, increased with drying (Fig. 5). Notice that if bornyl acetate were de-acetylated to produce borneol, that is sufficient to supply the increase in borneol (Fig. 5).

Variation in the leaf oil components is shown in Table 2. These results are very similar to the data based on mg/g DW basis (Table 1) and again show that the major effects were between 24 and 48 h of drying. It is interesting that, in the present study and others (Achak et al. 2008, 2009; Adams 2010, 2012, 2013), decomposition products have not been reported to occur during leaf drying. Thus, the characteristic taxon-specific terpene profile has not been lost.

The present study suggests that the changes reported to occur between fresh and 0.5 mo. (Adams, 2013), in fact, occurred when the leaves became dried to point of being brittle (24-48 h). Unfortunately, to preserve specimens for shipping and subsequent herbarium conservation, the leaves must be sufficiently dry so that mold and mildew do not grow.

For chemosystematic studies, the changes found in leaf oil composition during drying do not appear to prohibit their use for taxonomic purposes (Fig. 6). However, for studies of geographic variation (infra-specific), it seems prudent to use either all fresh or all dried materials.

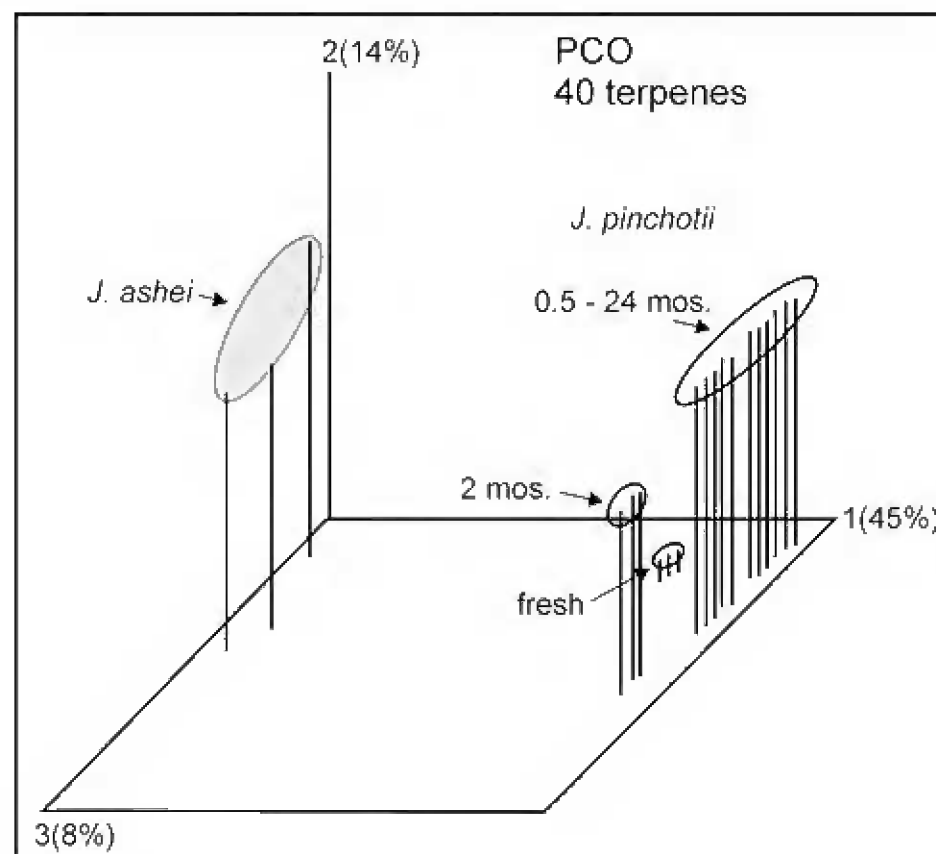


Figure 6. PCO ordination of *J. pinchotii* (oils from fresh and dried leaves) vs. *J. ashei* (fresh leaves). From Adams (2013).

### ACKNOWLEDGEMENTS

Thanks to Lawrence Cool and Billie Turner for reviews. Thanks to Tonya Yanke for lab assistance. This research was supported in part with funds from Baylor University.

### LITERATURE CITED

- Achak, N., A. Romane, M. Alifriqui and R. P. Adams. 2008. Effect of the leaf drying and geographic sources on the essential oil composition of *Juniperus thurifera* L. var. *africana* Maire from the Tensift-Al Haouz, Marrakech region. *J. Essential Oil Res.* 20: 200-204.
- Achak, N., A. Romane, M. Alifriqui and R. P. Adams. 2009. Chemical studies of the leaf essential oils of three species of *Juniperus* from Tensift Al Haouz-Marrakech Region (Morocco). *J. Essential Oil Res.* 21: 337-341.
- Adams, R. P. 1975. Statistical character weighting and similarity stability. *Brittonia* 27: 305-316.
- Adams, R. P. 1991. Cedar wood oil - analysis and properties. In *Modern Methods of Plant Analysis: Oils and Waxes*. Edits., H. F. Linskins and J. F. Jackson, pp. 159 - 173, Springer-Verlag, Berlin, Germany.
- Adams, R. P. 2007. Identification of essential oils by gas chromatography/ mass spectrometry, 4th edition. Allured Publ., Carol Stream, IL, USA.
- Adams, R. P. 2010. Chemosystematics of *Juniperus*: Effects of leaf drying on essential oil composition. *Phytologia* 92: 186-198.
- Adams, R. P. 2011. Chemosystematics of *Juniperus*: Effects of leaf drying on essential oil composition: II. *Phytologia* 93: 51-62.
- Adams, R. P. 2013. Chemosystematics of *Juniperus*: Effects of leaf drying on the essential oil composition of *Juniperus pinchotii*. *Phytologia* 95: 10-17.
- Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857-874.
- Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53: 326-338.
- Veldman, D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

Table 1 Comparison of leaf oils (mg/g x10 basis) for major components obtained from fresh leaves of *J. pinchotii* vs. leaves dried at 42-45° C for 4, 8, 12, 24, and 48 hours. ODW = oven dry wt. of extracted foliage. F sig = F ratio significance, P= 0.05 = \*; P= 0.01 = \*\*, ns = non significant, nt = not tested.

KI	Compound	Fresh	4 hr	8 hr	12 hr	24 hr	48 hr	F ratio	F sig
	yield mg/g ODW	16.4	14.5	14.7	16.6	15.7	12.9	7.97	**
924	$\alpha$ -thujene mg/g (x 10)	1.31	1.04	1.07	1.24	1.25	1.42	4.99	**
932	$\alpha$ -pinene	2.23	1.88	1.99	2.07	2.26	2.45	3.41	*
969	sabinene	42.55	40.30	44.10	48.90	46.67	37.19	11.64	**
988	myrcene	4.34	3.91	4.12	4.90	4.54	4.39	6.57	**
1014	$\alpha$ -terpinene	2.29	1.74	1.61	1.91	1.67	1.55	26.87	**
1024	limonene	5.82	5.22	5.51	6.13	5.87	5.22	6.19	**
1054	$\gamma$ -terpinene	3.59	2.82	2.65	2.42	2.66	2.45	8.78	**
1065	cis-sabinene hydrate	2.54	2.39	2.35	2.68	2.35	1.74	11.98	**
1086	terpinolene	1.63	1.45	1.47	1.54	1.56	1.42	3.61	*
1095	trans-sabinene hydrate	1.06	0.95	0.99	1.21	0.94	0.77	5.15	**
1141	camphor	42.23	37.02	33.21	41.42	36.92	27.25	18.11	**
1145	camphene hydrate	1.23	1.16	1.10	1.42	1.18	0.90	2.90	ns
1148	citronellal	1.23	1.85	2.57	2.06	1.64	1.49	3.86	*
1165	borneol	1.65	1.46	1.69	2.08	2.58	2.52	19.94	**
1166	coahuilensol	1.56	1.17	1.25	1.77	1.49	1.35	3.60	*
1174	terpinen-4-ol	9.15	6.69	5.74	5.12	5.95	4.64	4.66	*
1223	citronellol	5.92	5.89	6.39	6.63	5.87	4.13	12.46	**
1284	bornyl acetate	7.26	7.26	7.85	8.72	8.32	5.74	7.81	**
1548	elemol	8.62	8.75	8.09	9.06	9.40	6.91	4.33	*
1630	$\gamma$ -eudesmol	0.74	0.73	0.52	0.66	0.63	0.51	3.67	*
1649	$\beta$ -eudesmol	0.99	0.80	0.81	0.92	1.10	1.04	3.53	*
1652	$\alpha$ -eudesmol	1.07	0.80	0.81	0.89	1.10	0.97	5.41	**
1987	manoyl oxide	1.26	0.78	0.88	0.75	1.02	1.29	1.55	ns

KI = Kovats Index (linear) on DB-5 column.

Table 2. Comparison of leaf oils components (percent total oil basis) obtained from fresh leaves of *J. pinchotii* vs. leaves dried at 42-45° C for 4, 8, 12, 24, and 48 hours. ODW = oven dry wt. of extracted foliage. F sig = F ratio significance, P= 0.05 = \*; P= 0.01 = \*\*, ns = non significant, nt = not tested. t = trace, <0.1%.

KI	Compound	Fresh	4 h	8 h	12 h	24 h	48 h	F ratio	F sig
	percent yield (% ODW basis)	1.64	1.45	1.47	1.66	1.57	1.29	7.97	**
921	tricyclene	0.6	0.4	0.4	0.4	0.4	0.5	----	nt
924	$\alpha$ -thujene	0.8	0.7	0.8	0.8	0.8	1.1	19.57	**
932	$\alpha$ -pinene	1.4	1.3	1.4	1.3	1.5	1.9	10.20	**
946	camphene	0.7	0.6	0.5	0.5	0.5	0.5	----	nt
969	sabinene	25.9	27.8	30.0	29.5	29.8	28.9	45.21	**
974	$\beta$ -pinene	t	t	t	t	t	t	----	nt
988	myrcene	2.7	2.7	2.8	3.0	2.9	3.4	35.04	**
1002	$\alpha$ -phellandrene	t	t	t	t	t	t	----	nt
1014	$\alpha$ -terpinene	1.4	1.2	1.1	1.2	1.1	1.2	9.63	**
1020	p-cymene	t	t	t	t	t	t	----	nt
1024	limonene	3.5	3.6	3.8	3.7	3.8	4.1	10.52	**
1054	$\gamma$ -terpinene	2.0	2.09	1.8	1.5	1.7	1.9	10.19	**
1065	cis-sabinene hydrate	1.5	1.7	1.6	1.6	1.5	1.4	9.13	**
1086	terpinolene	1.0	1.0	1.0	1.0	1.0	1.1	1.93	ns
1098	trans-sabinene hydrate	0.7	0.7	0.7	0.7	0.6	0.6	2.53	ns
1118	cis-p-menth-2-en-1-ol	0.4	0.4	0.3	0.4	0.3	0.4	----	nt
1141	camphor	25.7	25.6	22.6	24.8	23.6	21.1	10.17	**
1145	camphene hydrate	0.8	0.8	0.8	0.9	0.8	0.7	1.37	ns
1148	citronellal	0.8	1.3	1.8	1.3	1.1	1.2	4.43	*
1165	borneol	1.0	1.0	1.2	1.3	1.7	2.0	89.21	**
1166	coahuilensol	0.9	0.8	0.9	1.1	1.0	1.1	3.59	*
1174	terpinen-4-ol	5.6	4.6	3.9	3.1	3.8	3.6	3.96	*
1186	$\alpha$ -terpineol	0.3	0.3	0.2	0.3	0.3	0.3	----	nt
1195	cis-piperitol	t	t	t	t	t	t	----	nt
1207	trans-piperitol	t	t	t	t	t	t	----	nt
1219	coahuilensol, me-ether	t	t	t	t	t	t	----	nt
1223	citronellol	3.6	4.0	4.4	4.0	3.8	3.2	6.14	**
1274	pregeijerene B	t	t	t	t	t	t	----	nt
1284	bornyl acetate	5.0	5.0	5.4	5.3	5.3	4.5	10.14	**
1298	carvacrol	t	t	t	t	t	t	----	nt
1374	$\alpha$ -copaene	t	t	t	t	t	t	----	nt
1451	trans-muurolo-3,5-diene	t	t	t	t	t	t	----	nt
1475	trans-cadina-1(6),4-diene	t	t	t	t	t	t	----	nt
1493	trans-muurolo-4,5-diene	t	t	t	t	t	t	----	nt
1493	epi-cubebol	t	t	t	t	t	t	----	nt
1500	$\alpha$ -muuroloene	t	t	t	t	t	t	----	nt
1514	cubebol	t	t	t	t	t	t	----	nt
1522	$\delta$ -cadinene	t	t	t	t	t	t	----	nt
1548	elemol	5.3	6.0	5.5	5.5	6.0	5.4	1.46	ns
1559	germacrene B	0.3	t	t	t	t	0.4		
1627	1-epi-cubenol	t	t	0.3	t	t	0.3		
1630	$\gamma$ -eudesmol	0.5	0.5	0.4	0.4	0.4	0.4	3.20	*
1649	$\beta$ -eudesmol	0.6	0.6	0.6	0.6	0.7	0.8	8.60	**
1652	$\alpha$ -eudesmol	0.7	0.6	0.6	0.5	0.7	0.8	9.43	**
1670	bulnesol	t	t	t	t	t	t	----	nt
1987	manoyl oxide	0.8	0.5	0.6	0.5	0.7	1.0	3.72	*
2055	abietatriene	t	t	t	t	t	t	----	nt
2087	abietadiene	0.2	t	t	t	t	0.1	----	nt
2298	4-epi-abietal	0.4	0.1	0.2	0.1	0.2	0.3	----	nt
2312	abieta-7,13-dien-3-one + abietal	0.5	0.3	0.2	0.2	0.3	0.3	----	nt